

## COMBINING PHOSPHODIESTERASE-4 AND PHOSPHODIESTERASE-5 INHIBITORS IN A RAT MODEL OF OVERACTIVE BLADDER

### Hypothesis / aims of study

Overactive bladder (OAB) is a prevalent disorder that greatly reduces quality of life; however, current treatments are often discontinued due to side effects (1). The presence of both Phosphodiesterase type 4 (PDE4) and Phosphodiesterase type 5 (PDE5) enzymes in the bladder suggest the use of their inhibitors as a therapeutic agent for OAB. Phosphodiesterase type 4 inhibitors (PDE4i) and Phosphodiesterase type 5 inhibitors (PDE5i) have both been shown to decrease calcium influx in afferent nerves (2). This would reduce afferent nerve firing, resulting in fewer muscle contractions. PDE4i can be used to treat OAB but PDE4 is also highly expressed in brain and other tissues, producing side effects.(3) PDE5i is less effective in treating OAB but has fewer side effects. The goal of this study was to determine if administration of PDE5i with PDE4i increases effectiveness and/or enables reduction of the dose of PDE4i to reduce symptoms of and decrease pathology of partial bladder outlet obstruction (PBOO) in rats.

### Study design, materials and methods

This study was designed to provide significance with  $p < 0.05$  at 80% power and required 12 animals per group. Sixty female age-matched Sprague-Dawley rats weighing 225-250g, underwent PBOO and were treated by gavage with PDE4i (1mg/kg, n=12), PDE5i (10mg/kg, n=12), high dose combination treatment (PDE4i 1mg/kg, PDE5i 10mg/kg, n=12), low dose combination treatment (PDE4i 0.2mg/kg, PDE5i 10mg/kg, n=12), or sham treatment (vehicle :HEC 1%/Tween 80 0.25%/Antifoam 0.05% ) n=12). An additional 12 age-matched female rats underwent sham PBOO and received sham treatment. PBOO was performed by exposing the bladder and urethra, placing a 1.0 mm rod next to the urethra, and tying a suture around both the urethra and the rod to create a loose ligature when the rod was removed. Sham PBOO was performed similarly, without tying the suture. Treatments were started the day following the surgery and rats were gavaged daily for thirty days by researchers blinded to the treatment groups. Twenty-eight days after surgery the animals underwent suprapubic bladder catheter implantation. Two days later the rats underwent conscious and anesthetized cystometry (urethane anesthesia, 1.2g/kg intraperitoneally). After functional testing the animals were euthanized and the bladder was emptied and weighed. It was then filled with 1 ml formalin, embedded in paraffin, sectioned, and Masson's trichrome stained for qualitative histological assessment. Pressure and voiding data was digitized and collected (at 10 samples/second). Number of non-voiding contractions in each filling cycle was defined as at least a 20% rise in pressure from baseline in the absence of a void or urine leakage. These were counted and were divided by the duration of the void to calculate the number of nonvoiding contractions/minute. Threshold pressure was determined by selecting the lowest pressure just prior to the pressure increase for voiding. Quantitative outcomes were compared using a One way ANOVA on Ranks followed by a Dunn's test with  $p < 0.05$  indicating a statistically significant difference between experimental groups. Quantitative data is presented as mean  $\pm$  standard error of the mean

### Results

Weight of the lower urinary tract after PBOO, a measure of the pathology of PBOO, was significantly increased with sham treatment (1.2 g  $\pm$  0.2 mg), PDE4i alone (1.2 g  $\pm$  0.1 mg) and PDE5i alone (1.0 g  $\pm$  0.1 mg) compared to that of sham PBOO rats (0.6 g  $\pm$  0.1 mg). In contrast, neither combination treatment demonstrated a significant increase in weight, indicating reduced pathology after PBOO with these treatments. A significant increase in nonvoiding contractions per void during conscious cystometry was observed with sham treatment (18.8  $\pm$  5.9 contractions/void) and PDE5i treatment alone (27.2  $\pm$  8.6 contractions/void) after PBOO compared to sham PBOO rats (12.4  $\pm$  4.3 contractions/void), indicating increased OAB symptoms in these animals. There was no significant difference in number of nonvoiding contractions/void between PDE4i alone (16.9  $\pm$  3.7 contractions/void) and combination treatment (low dose: 18.8  $\pm$  3.7 contractions/void; high dose: 20.4  $\pm$  4.9 contractions/void) compared to sham PBOO animals, indicating normalization of OAB symptoms in these groups. A significant increase in nonvoiding contractions/minute was observed during anesthetized cystometry after PBOO with either sham treatment (1.8  $\pm$  0.2 contractions/min), PDE5i alone (1.4  $\pm$  0.2 contractions/min) or low dose combination treatment (1.7  $\pm$  0.2 contractions/min) compared to sham PBOO (0.3  $\pm$  0.2 contractions/min), indicating increased OAB symptoms. No significant differences from sham PBOO were observed after PBOO with PDE4i treatment alone (0.9  $\pm$  0.3 contractions/min) or with high dose combination treatment (1.0  $\pm$  0.2 contractions/min). Threshold pressure for voiding during anesthetized cystometry was significantly decreased with high dose combination treatment (7.7  $\pm$  0.6 cm H<sub>2</sub>O) compared to PBOO with sham treatment (14.4  $\pm$  2.5 cm H<sub>2</sub>O), PDE5i alone (12.6  $\pm$  1.0 cm H<sub>2</sub>O) and low dose combination treatment (13.2  $\pm$  1.4 cm H<sub>2</sub>O). Sham PBOO (9.5  $\pm$  0.9 cm H<sub>2</sub>O) threshold pressures were not significantly different compared to high dose combination treatment or PDE4i alone treatment (9.2  $\pm$  2.8 cm H<sub>2</sub>O). Less smooth muscle fragmentation was observed in the bladder of PBOO animals treated with PDE4i alone or high dose combination treatment compared to sham treatment, PDE5i alone, or low dose combination treatment

### Interpretation of results

PDE5i alone had no significant effect on the symptoms of OAB or pathology of PBOO at the dose used. PDE4i alone was not effective in reducing lower urinary tract weight after PBOO. Both combination treatments reduced nonvoiding contractions per void during conscious cystometry. These results taken together, suggest that the combination treatments provide the greatest therapeutic effect to reduce pathology of PBOO. High dose combination treatment more effectively normalized nonvoiding contractions per minute and threshold pressure than low dose combination treatment after PBOO, demonstrating that the high dose combination treatment is more effective in reducing the symptoms of OAB. This is supported by the reduced muscle

fragmentation observed in the high dose combination treatment. The low dose PDE4i combination treatment may be more effective with a higher dose of PDE5i.

#### Concluding message

This study shows that the high dose PDE4i/PDE5i combination treatment is more effective than the low dose PDE4i combination or PDE4i alone as a treatment for OAB

#### References

1. Abrams P, Cardozo L, Fall M, et al. The standardisation of terminology in lower urinary tract function: report from the standardisation sub-committee of the International Continence Society. *Urology* 2003 Jan;61:37-49.
2. Kanai A, Zabbarova I, Oefelein M, Radziszewski P, Ikeda Y, Andersson KE. Mechanisms of action of botulinum neurotoxins, beta3-adrenergic receptor agonists, and PDE5 inhibitors in modulating detrusor function in overactive bladders: ICI-RS 2011. *Neurourol Urodyn* 2012 Mar;31:300-308.
3. Richter W, Menniti FS, Zhang HT, Conti M. PDE4 as a target for cognition enhancement. *Expert Opin Ther Targets* 2013 Sep;17:1011-1027.

#### Disclosures

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